A 5-hydroxytryptamine receptor antagonist, sarpogrelate, reduces renal tubulointerstitial fibrosis by suppressing PAI-1

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1Department of Nephrology and Endocrinology, The University of Tokyo, Tokyo, Japan; 2Department of Emergency and Critical Care Medicine, The University of Tokyo, Tokyo, Japan; 3Department of Apheresis and Dialysis, The University of Tokyo, Tokyo, Japan; 422nd Century Medical and Research Center, University Hospital, The University of Tokyo, Tokyo, Japan; 5Department of Urology, Nagoya University, Nagoya, Japan; 6CMIC Company, Limited, Tokyo, Japan; and 7Japan Science and Technology Agency/Japan International Cooperation Agency (JST/JICA), Science and Technology Research Partnership for Sustainable Development (SATREPS), Tokyo, Japan

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Hamasaki Y, Doi K, Maeda-Mamiya R, Ogawara E, Katagiri D, Tanaka T, Yamamoto T, Sugaya T, Nangaku M, Noiri E. A 5-hydroxytryptamine receptor antagonist, sarpogrelate, reduces renal tubulointerstitial fibrosis by suppressing PAI-1. Am J Physiol Renal Physiol 305: F1796–F1803, 2013. First published October 9, 2013; doi:10.1152/ajprenal.00151.2013.—A selective 5-hydroxytryptamine (5-HT) 2A receptor antagonist sarpogrelate (SG) blocks serotonin-induced platelet aggregation. It has been used clinically for the treatment of peripheral arterial disease. SG might be able to improve chronic ischemia, which contributes to renal fibrosis progression by maintaining renal microcirculation. This study investigated whether SG suppresses renal fibrosis. C57BL/6 mice fed a 0.2% adenine-containing diet for 6 wk developed severe tubulointerstitial fibrosis with kidney dysfunction. Subsequent SG treatment (30 mg·kg⁻¹·day⁻¹) for 4 wk improved these changes significantly by increasing peritubular blood flow in the fibrotic area, as evaluated by intravital microscopy and decreasing fibrin deposition. Urinary L-type fatty acid-binding protein, up-regulated by renal hypoxia, was also reduced by SG. Additionally, results showed that mRNA expression of plasminogen activator inhibitor-1 (PAI-1), which is known to promote fibrosis by mediating and enhancing transforming growth factor (TGF)-β1 signaling, was suppressed by SG treatment in the kidney. In vitro experiments using cultured murine proximal tubular epithelial (mPtox) cells revealed that incubation with TGF-β1 and 5-HT increased PAI-1 mRNA expression; SG significantly reduced it. In conclusion, SG reduces renal fibrosis not only by the antithrombotic effect of maintaining peritubular blood flow but also by suppressing PAI-1 expression in renal tubular cells.

L-FABP; PAI-1; peritubular blood flow; sarpogrelate; tubulointerstitial fibrosis

THE INCIDENCE AND PREVALENCE of chronic kidney disease (CKD) continue to increase, entailing poor outcomes and high costs (3, 49). CKD is well known to contribute strongly to cardiovascular disease and high mortality (7, 19, 33, 42). Although CKD progresses slowly, it eventually evolves to end-stage renal disease, which necessitates renal replacement therapy such as dialysis or kidney transplantation (37). The best predictive factor for progression of CKD to end-stage renal disease is not the etiology of glomerular injury but the degree of tubulointerstitial damage such as fibrosis and inflammatory cell infiltration (26, 31, 37, 39). Tubulointerstitial fibrosis is a common pathological change occurring in association with chronic kidney damage (38).

5-Hydroxytryptamine (5-HT), synthesized from L-tryptophan and stored in platelets, is subsequently secreted, promoting not only platelet aggregation but also proliferation of vascular smooth muscle cells, leading to vascular occlusion at the site of vascular injury (51). The 5-HT2A receptor, one of seven 5-HT receptor subfamilies, mediates the effect of 5-HT on platelet aggregation and vasoconstriction. It is known that the system for 5-HT synthesis is present in the kidney and that 5-HT signaling through the 5-HT2A receptor accelerates the expression of transforming growth factor (TGF)-β1 in cultured cells derived from the kidney (9, 17, 44, 53). Sarpogrelate (SG), a selective antagonist of the 5-HT2A receptor, is widely used for the treatment of peripheral arterial disease and cardiovascular disease because of its vasodilation and antiplatelet effects (12, 40). SG in combination with antidiabetic agents increases plasma levels of adiponectin, an adipokine, in diabetic patients with peripheral arterial disease (54). Although renoprotective effects of SG in animal models of glomerulonephritis and diabetic nephropathy have been described in several reports (6, 16, 17, 22), the protective effects of SG on renal fibrosis or renal tubular cells remain unclear.

Plasminogen activator inhibitor-1 (PAI-1) is a 50-kDa single-chain glycoprotein with serine proteinase inhibitor activity. PAI-1 inhibits fibrinolysis and plays a role in the pathogenesis of thrombosis in diseases such as coronary artery disease (23). During the past decade, many studies using various animal models have demonstrated that PAI-1 is an important downstream effector of TGF-β1 and that PAI-1 promotes tissue fibrosis (5, 10, 24). PAI-1, which serves a crucial role in promoting renal fibrosis, is anticipated as a therapeutic target to prevent CKD (5). Some reports have described a link between 5-HT signaling mediated by the 5-HT2A receptor and PAI-1. PAI-1 expression in vascular endothelial cells, which is induced by 5-HT stimulation, is improved by treatment with SG (18). Furthermore, PAI-1 expression is reduced by the administration of SG or knockdown of the 5-H2A receptor in adipocytes (50). These findings indicate the possibility that antagonism of 5-HT2A receptors decreases PAI-1 expression. However, it remains unknown whether SG improves renal fibrosis by reducing PAI-1 expression in the kidney.

This study examined the protective effect of SG on a mouse renal tubulointerstitial fibrosis model, which is induced by feeding mice an adenosine-containing diet (Ad) for several weeks.
We further examined peritubular blood flow and urinary L-type fatty acid-binding protein (L-FABP) excretion to evaluate renal ischemia and hypoxia, as reported previously (15, 48, 56). We also performed in vitro analysis with murine proximal tubular epithelial (mProx) cells to investigate whether PAI-1 expression in proximal tubular epithelial cells is upregulated by 5-HT stimulation and improved by SG treatment.

MATERIALS AND METHODS

Animal experiments. Eight-week-old male wild-type C57BL/6 mice were used in this experiment. Male heterozygous hL-FABP transgenic (Tg) mice (C57BL/6 background), weighing 25–35 g, were also used to evaluate urinary hL-FABP. The engineering of hL-FABP Tg mice was described previously (15, 56). All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NIH Publication No. 85-23, 1985) and were approved by The University of Tokyo Institutional Review Board.

Both the SG-treated group (Ad+ SG group) and the untreated group (Ad group) were fed a 0.2% wt/wt adenine-containing diet (Oriental Yeast, Tokyo, Japan) dissolved in distilled water for 4 wk from 2 wk after starting the adenine-containing diet, similarly to a process used in a previous study demonstrating the effect of a drug against tubulointerstitial fibrosis (11). Based on doses of SG administered to various animal models in previous studies (13, 34), we chose the dosage of SG for the present study to be equal to those used in previous studies (30 mg·kg⁻¹·day⁻¹). The dosage of SG was based on evidence that SG suppresses platelet aggregation and formation of platelet-rich thrombus by blockade of 5-HT2A receptors in rodents (12). To evaluate the dose-response effect of SG to suppress chronic kidney injury induced by an adenine-containing diet, three different dosages (3, 30, and 300 mg·kg⁻¹·day⁻¹) were evaluated. The SG solution concentration was adjusted to give mice 3, 30, and 300 mg/kg SG daily based on the amount of drinking water consumed. The Ad group was given distilled water only. The normal-diet group (N group) was fed a normal diet and given water for 6 wk. The normal diet was purchased from Oriental Yeast. Its components were identical to those of the normal diet and given water for 6 wk after starting the adenine-containing diet, fixed in 10% buffered formalin, and embedded in paraffin. Sections (2 μm thick) were cut and used for Masson’s trichrome (MT) and immunohistochemistry. After antigen retrieval with 100 μM protease K for 30 min, an anti-human fibrin-fibrinogen antibody (Dako Denmark, Glostrup, Denmark) was used for fibrin immunohistochemical staining, as previously described (57). After microwave boiling of tissue for 10 min in 10 mM sodium citrate buffer (pH 6.0), immunostaining for PAI-1 and type IV collagen was conducted respectively using a rabbit polyclonal anti-PAI-1 antibody (H-135; Santa Cruz Biotechnology, Santa Cruz, CA) and type IV collagen antibody (Abcam, Cambridge, UK). Immunohistochemistry for F4/80 and α-smooth muscle actin (α-SMA) was performed as described previously (47). The interstitial fibrotic area, fibrinogen-positive area, F4/80-positive macrophages, α-SMA-positive area, and type IV collagen-positive area were evaluated using a computer-aided evaluation program (AIS; Imaging Research, St. Catharines, ON) as described previously (47).

Measurement of peritubular blood flow. Peritubular capillary images of mice were obtained using an intravital video charge-coupled device (CCD) 4 wk after the start of the adenine-containing diet. Renal capillary blood flow was visualized and measured according to a previously reported method (55, 56).

Measurement of urinary human L-FABP by ELISA. Male human L-FABP transgenic mice were kept in glass-shielded metabolic cages for 4 wk after starting the adenine-containing diet for 24 h to collect urine. To evaluate proximal tubular injury, urinary human L-FABP was measured using a sandwich ELISA kit (CMIC, Tokyo, Japan) as described previously (32, 56). Urinary human L-FABP levels are expressed as the ratio to the urinary creatinine concentration measured using a commercial kit (Nescoat VLI CRE, Alphresa Pharma, Osaka, Japan). Measurements were performed in duplicate.

Cell culture. The stable C57BL/6 mice proximal tubular epithelial cell line mProx was obtained as described previously (56) and cultured in DMEM (containing 10% FBS, 100 μM penicillin G, and 100 μg/ml streptomycin; Gibco, Carlsbad, CA) in a humidified atmosphere containing 5% CO₂ at 37°C. The mProx cells were seeded on 12-well culture plates in complete medium containing 10% FBS. After mProx cells were incubated with serum-free medium with 10 μM 5-HT and 10 μM SG for 3 h. Based on the previous study showing dose-dependent effects of 5-HT and cytotoxicity of SG by the concentration of more than 10 μM, we decided on the concentration of 5-HT and SG in this study (6). PAI-1 mRNA expression in mProx cells was increased significantly after 3 h incubation with 5-HT following TGF-β1 stimulation when it was evaluated in the different time courses (3, 6, 12 h) in our preliminary study. Therefore, we decided to incubate mProx cells with 5-HT and SG for 3 h. Cells were washed with PBS three times and lysed using TRizol Reagent (Invitrogen, Carlsbad, CA) to extract total RNA.

Quantitative PCR analysis of PAI-1, monocyte chemoattractant protein-1, and 5-HT2A receptor expression. Total RNA isolated from the harvested kidneys or mProx cells were reverse transcribed to cDNA using recombinant Moloney leukemia virus reverse transcriptase and random hexamers (High Capacity cDNA Reverse Transcription Kit; Applied Biosystems, Carlsbad, CA). Transcripts encoding PAI-1 and monocyte chemoattractant protein-1 (MCP-1) were measured using TaqMan real-time quantitative PCR with the Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA) and TaqMan Universal PCR master mix (Applied Biosystems) as described previously (47). The TaqMan probes and primers for PAI-1 (assay identification number Mm01310498_m1), and MCP-1 (assay identification number Mm00441242_m1) were assay-on-demand gene expression products (Applied Biosystems). To normalize for variance in loaded cDNA, 18S ribosomal RNA (18S rRNA) was amplified in a separate tube using TaqMan-based quantitative PCR. Probe primers for 18S rRNA were obtained from Applied Biosystems. Standard curves were prepared for each gene and the 18S rRNA in each experiment to normalize the relative expression of the genes of interest to the 18S rRNA control.

To investigate whether the 5-HT2A receptor is expressed in mProx cells derived from C57BL/6 mice and kidneys, transcripts encoding the 5-HT2A receptor and GAPDH were amplified by PCR. Then, PCR products were electrophoresed in 2% agarose 1× TAE gel. The sequences of primers are as follows: murine 5-HT2A receptor (forward: 5′-CCACGCGGCTCCATCCACAGAG-3′; reverse: 5′-ACCACTTACACAAACACACACAGACAC-3′), murine GAPDH (forward: 5′-ACCACTTACACAAACACACACAGACAC-3′; reverse: 5′-TCCACACACCGTTGCTGTA-3′). PCR products were visualized using GelGreen (Biotium, Hayward, CA) and a CCD camera system (LAS-4000 mini; Fuji Photo Film, Tokyo, Japan).

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RESULTS

Effects of SG on renal function and histopathological changes in adenine-induced mouse tubulointerstitial injury model. To evaluate dose-response effects of SG for protection of the kidney in an adenine-induced mouse tubulointerstitial injury model, mice were treated with three different doses of SG. Both BUN and Cre concentrations in the 30 mg·kg\(^{-1}\)·day\(^{-1}\) SG treatment group [middle-dose (M) group] were significantly lower than in the no treatment group (Ad group), although those in the 3 mg·kg\(^{-1}\)·day\(^{-1}\) SG treatment [low-dose (L)] group and the 300 mg·kg\(^{-1}\)·day\(^{-1}\) SG treatment [high-dose (H)] group were not (Fig. 1, A and B). Fibrin deposition in the kidneys of the M group was significantly reduced compared with that in the Ad group. However, those in the L and H groups were not (Fig. 1, C and D). According to these results, we decided to pursue further experiments with the dose of 30 mg·kg\(^{-1}\)·day\(^{-1}\).

The body weight of mice in the adenine diet group was significantly lower than the normal diet group from 2 wk after starting the adenine-containing diet (Fig. 2A). BUN levels in the adenine diet group were significantly higher than those of the normal-diet (N) group 2 wk after the start of the adenine diet [N group, 28.2 ± 0.5 mg/dl (n = 5); adenine diet group, 62.1 ± 3.7 mg/dl (n = 20)]. Mice in the adenine diet group were divided equally into two groups at 2 wk: the vehicle only (Ad) group and the SG treatment (Ad+SG) groups. The levels of BUN and Cre in the Ad+SG group (n = 10) were significantly lower than the Ad group (n = 10) at 4 and 6 wk (Fig. 2, B and C), although SG had no effect on the body weight of mice in these two groups (Fig. 2A). Pathological evaluation showed remarkable interstitial fibrosis, irregular renal tubular dilatation, and deposition of fibrin in the kidney of mice fed the adenine-containing diet at 6 wk (Fig. 3A). Quantitative analyses of the fibrotic area and fibrin-positive area revealed that SG improved these histopathological changes (Fig. 3, B and C). PAI-1 expression in tubular epithelial cells was increased in the adenine diet group and was reduced significantly by SG treatment (Fig. 3, A and D). F4/80-positive inflammatory cell (macrophage) infiltration in the interstitium was increased by adenine administration and reduced by SG treatment (Fig. 3, A and E). The levels of expression of both α-SMA and type IV collagen were significantly increased in the kidneys of mice fed the adenine-containing diet and were improved by SG treatment (Fig. 3, A, F, and G).

Effects of SG on PAI-1, MCP-1, and TGF-β1 expression in the kidney. The mRNA expressions of PAI-1, MCP-1, and TGF-β1 in the kidney were increased significantly by adenine administration. PAI-1 expression was reduced significantly by SG treatment (Fig. 4A). The expression of MCP-1 was not changed significantly by SG treatment (Fig. 4B), whereas TGF-β1 tended to be decreased by SG with no statistical significance (Fig. 4C).

Evaluation of peritubular blood flow in the kidney. We evaluated peritubular blood flow in an adenine-induced renal injury model using intravital video CCD images at 4 wk. In the Ad group, peritubular blood flow was significantly decreased compared with that of the N group. Animals treated with SG showed significantly higher blood flow than did the untreated animals (Fig. 5A).

Effects of SG on urinary excretion of L-FABP. Reportedly, urinary L-FABP levels in the adenine-induced mouse tubulointerstitial injury model reflect the degree of fibrosis (47). Urinary secretion of L-FABP was apparently induced by hypoxia (4). In the current study, urinary L-FABP level was increased in the Ad group at 4 wk after the beginning of the adenine-containing diet. SG reduced urinary L-FABP levels significantly (Fig. 5B).

Expression of 5-HT receptors and PAI-1 in renal proximal tubular epithelial cells. We conducted in vitro experiments using mProx cells. In mProx cells, expression of the 5-HT2A
receptor, for which SG has high affinity, was confirmed by RT-PCR as in the mouse kidney (Fig. 6A). An in vitro model of tubular injury was induced by incubating mProx cells with TGF-β1 (5 ng/ml) and 5-HT (10 μM). On quantitative realtime PCR, PAI-1 expression was increased significantly by TGF-β1 and was increased further by addition of 5-HT (Fig. 6B). In our preliminary experiments, mRNA expression of PAI-1 was not increased significantly in the mProx cells stimulated by 5-HT without TGF-β1 compared with the control cells. SG treatment significantly reduced PAI-1 expression in mProx cells incubated with TGF-β1 and 5-HT (Fig. 6B).

DISCUSSION

A selective 5-HT2A receptor antagonist, SG, is used clinically for prevention of platelet aggregation and subsequent thrombosis formation. This study demonstrated that SG ameliorated renal dysfunction and pathologic changes in fibrosis in a mouse tubulointerstitial injury model. SG reduced fibrin deposition and maintained renal microcirculation. Moreover, SG significantly suppressed the increase in PAI-1 expression levels in the fibrotic kidney. We further demonstrated that PAI-1 expression was upregulated synergistically by TGF-β1 and 5-HT in mProx cells and that it was reduced significantly by SG treatment. Results show for the first time that suppression of PAI-1 by SG can ameliorate the progression of renal fibrosis.

In this study, we investigated renoprotective effects of SG using an adenine-induced tubulointerstitial fibrosis model. The adenine-induced chronic renal failure, originally described in rats, has been regarded as a useful animal model of CKD because it resembles human uremic features such as renal dysfunction, severe anemia, and secondary hyperparathyroidism (1, 45, 59). An adenine-induced CKD model in C57BL/6 mice was also developed, and several experimental reports using this model using mice have been published recently (41, 46, 47). Renal fibrosis is another characteristic of this model. Mice fed with an adenine-containing diet show renal fibrosis as severe as that in mice with unilateral ureteral obstruction (UUO) (11, 29). The mechanism of renal injury is regarded as follows. Adenine in the chow is oxidized to 2,8-dihydroxyadenine by xanthine dehydrogenase after intestinal absorption. Then, 2,8-dihydroxyadenine will be precipitated in renal tubules because of its low solubility. Deposition of 2,8-dihydroxyadenine crystals degenerates renal tubular epithelial cells and causes inflammatory injury with subsequent fibrotic changes.

In CKD, the uptake of synthesized 5-HT into platelets is decreased and the plasma concentration of 5-HT is increased (27), which suggests that increased 5-HT concentration in the systemic circulation might contribute to the progression of CKD. Reportedly, SG can improve glomerular injury through several different mechanisms such as reducing glomerular platelet activation in a rat diabetic nephropathy model (22), or inhibiting 5-HT1-induced type IV collagen secretion and suppressing mitogenic signaling in cultured mesangial cells (6, 17). Reportedly, 5-HT signaling through the 5-HT2A receptor accelerates the expression of TGF-β1 via the PKC-MEK-ERK pathway in mesangial cells (9, 17). Are mesangial cells in glomeruli the only target of the 5-HT-related pathway? In this study, we used a mouse tubulointerstitial injury model by administering adenine, in which no obvious glomerular lesion such as sclerosis or mesangial expansion was observed (47). In this study, although fibrin deposition and PAI-1 expression were observed in some glomeruli in addition to tubulointerstitial injury of the adenine diet group, no glomerular change induced by chronic glomerular damage such as global sclerosis was observed. Tubulointerstitial fibrosis induced by chronic tubulointerstitial injury was diffuse and severe. Renal injury induced by adenine is thought to derive from renal tubular occlusion with 2,8-dihydroxyadenine crystals (59). Therefore, it is reasonable to infer that glomerular change follows tubulointerstitial injury in this model. To improve tubulointerstitial injury by SG treatment in this animal model, some mechanism of action other than glomerular protection by SG is suggested.

PAI-1 is regarded as playing a critical regulatory role in fibrosis. Although elevated PAI-1 promotes fibrosis, decreased PAI-1 reduces it, but the mechanism of these effects remains elusive (25). Reportedly, tubulointerstitial fibrosis was worsened by PAI-1 overexpression and was ameliorated by knocking down of PAI-1 in an ureteral obstruction-induced renal fibrosis model (28, 35). PAI-1 expression is induced by TGF-β1 through up-regulation of reactive oxygen species (ROS), but TGF-β1 expression is accelerated by PAI-1 (5, 10). Therefore, PAI-1 will not only act as an effector of TGF-β1 but will also constitute a positive feedback loop together with TGF-β1 in renal fibrosis progression (43). PAI-1 expression was increased not only by TGF-β1 but also by 5-HT stimulation in endothelial cells or adipocytes (18, 50). In this study, the levels of PAI-1 in mProx cells incubated with 5-HT and TGF-β1 in mProx cells were measured at 0, 2, 4, and 6 wk. BW, BUN, and Cre were presented. BW, BUN, and Cre were measured at 0, 2, 4, and 6 wk (A). N, normal diet; Ad, adenine diet; Ad + SG, adenine diet and sarpogrelate treatment. Values are means ± SE; n = 5 in the N group and n = 10 in the Ad group and Ad + SG group each. #P < 0.05 vs. Ad group at the same time point.

Fig. 2. Effect of sarpogrelate on mouse adenine-induced tubulointerstitial injury model. Course of body weight (BW), blood urea nitrogen (BUN), and plasma creatinine (Cre) levels are presented. BW, BUN, and Cre were measured at 0, 2, 4, and 6 wk (A–C). N, normal diet; Ad, adenine diet; Ad + SG, adenine diet and sarpogrelate treatment. Values are means ± SE; n = 5 in the N group and n = 10 in the Ad group and Ad + SG group each. #P < 0.05 vs. Ad group at the same time point.
Fig. 3. Renal pathological findings in mouse adenine-induced tubulointerstitial injury model at 6 wk. Renal histological changes evaluated using Masson’s trichrome (MT) staining and immunohistochemistry are shown (A). Original magnification: ×200. Fibrotic area in MT stain (B), positive staining area of fibrin (C), plasminogen activator inhibitor-1 (PAI-1; D), F4/80 (E), α-smooth muscle actin (SMA; F), and type IV collagen (G) were quantified. N, normal diet; Ad, adenine diet; Ad+SG, adenine diet and sarpogrelate treatment. Values are means ± SE; n = 5 in the N group and n = 10 in the Ad group and Ad+SG group each. #P < 0.05.
A selective 5-HT2A receptor antagonist, SG, reduced PAI-1 expression both in vivo and in vitro. Additionally, we confirmed 5-HT2A receptor expression in mProx cells, as in the murine kidney (Fig. 6A) (36, 53). These observations indicated that PAI-1 expression triggered by TGF-β1 was accelerated by 5-HT via the 5-HT2A receptor.

We first demonstrated that SG ameliorated adenine-induced tubulointerstitial fibrosis in mice and that it significantly reduced the renal expression of PAI-1, which is known as a key molecule in renal fibrosis progression. Renal PAI-1 expression has been shown to be increased not only in chronic renal injury animal models such as UUO, 5/6 nephrectomy, and glomerulonephritis but also in human CKD patients (5, 8, 28). PAI-1 accelerates chronic kidney injury in various animal models. Therefore, it is possible that suppression of PAI-1 by SG treatment at least partly improves renal fibrosis in other animal models as well as it does in the adenine-induced renal fibrosis model. Recently, SG treatment was reported to improve liver fibrosis accompanied by a reduction in TGF-β and α-SMA; both were common molecules for which expressions were increased in renal fibrosis progression (20). Further evaluation must be done to confirm the renal-protective effect of SG in different animal models.

This study showed that peritubular blood flow in a fibrotic kidney was reduced significantly compared with that in a normal kidney. A relationship between microcirculation in the kidney and renal fibrosis has been suggested. Reportedly, renal microcirculation is reduced before progression of renal fibrosis.

![Fig. 4. mRNA expressions of PAI-1, monocyte chemoattractant protein-1 (MCP-1), and transforming growth factor (TGF)-β1 in mouse adenine-induced tubulointerstitial injury model. Expressions of PAI-1 (A), MCP-1 (B), and TGF-β1 (C) were evaluated using quantitative real-time PCR. N, normal diet; Ad, adenine diet; Ad+SG, adenine diet and sarpogrelate treatment. Values are means ± SE; n = 5 in the N group and n = 10 in the Ad group and Ad+SG group each. #P < 0.05.]

![Fig. 5. Peritubular blood flow and urinary L-type fatty acid-binding protein (L-FABP) in mouse adenine-induced tubulointerstitial injury model. Peritubular blood flow was measured in the kidney of mice by intravital charge-coupled device (CCD) videography (A). Urinary L-FABP was measured using 24-h collected urine at 4 wk: N, normal diet; Ad, adenine diet; Ad+SG, adenine diet and sarpogrelate treatment. Values are means ± SE; n = 5 in each group. #P < 0.05.]

![Fig. 6. Expressions of mRNA of 5-hydroxytryptamine (5-HT) 2A receptor and PAI-1 in mProx cells. Expression of 5-HT2A receptor in mProx cells and mouse kidney was evaluated by RT-PCR (A). PAI-1 expression in mProx cells was induced synergistically by 5-HT and TGF-β1 (B, black bar), and sarpogrelate suppressed PAI-1 expression (B, gray bar). Values are means ± SE; n = 2 (A) and n = 8–12 obtained from more than 3 independently conducted experiments in each group (B). #P < 0.05.]

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(2), and that protection of endothelial cells increases renal cortical blood flow and reduces renal fibrosis in UUO (21). Reportedly, tubulointerstitial hypoxia, derived from impaired blood flow in the kidney, might induce tubulointerstitial fibrosis and reduction of peritubular capillaries; the fibrosis, in turn, will impair the tubulointerstitial oxygen supply (26, 30). Therefore, reduced renal blood flow and tubulointerstitial fibrosis constitute a vicious cycle that accelerates renal injury. Although the 5-HT receptor antagonist ritanserin reportedly improved impairment of renal blood flow autoregulation by cyclosporine (52), no report in the relevant literature has described that SG, a selective 5-HT2A receptor antagonist, improves peritubular blood flow and renal fibrosis. SG might preserve blood flow directly by inhibiting aggregation of the platelets which causes thrombosis. Actually, results from our study showed that fibrin deposition in the kidney was reduced significantly by SG. SG apparently suppresses the vicious cycle between renal blood flow and renal fibrosis progression by ameliorating renal fibrosis via reducing TGF-β1 or PAI-1 expression in the expression. Therefore, SG treatment might preserve microcirculation in the kidney by maintaining the normal structure of the peritubular capillary network.

Increased urinary L-FABP level showed significant correlation with decreased peritubular capillary blood flow and progression of tubulointerstitial fibrosis in human and animal models (47, 48, 56). Of note, urinary L-FABP levels in CKD patients were correlated to the progression of CKD and the degree of proteinuria (14). The promoter of the L-FABP gene contains the hypoxia-responsive element. Therefore, hypoxic injury to renal tubular cells can be detected by measurement of urinary L-FABP (32, 56, 58). Moreover, a recent study demonstrated that urinary secretion of L-FABP is induced by renal hypoxia (4). In this study, urinary L-FABP concentration was increased in the Ad group and was reduced significantly by SG treatment accompanied by improvement of peritubular blood flow and tubulointerstitial fibrosis. Chronic hypoxia caused by decreased peritubular capillary blood flow attributable to tubulointerstitial damage might upregulate the expression of L-FABP.

In conclusion, SG prevented the progression of renal fibrosis by multiple pathways in a mouse tubulointerstitial injury model. Among those pathways, suppressing PAI-1 expression by SG was further confirmed by in vitro analysis. Results show that 5-HT decreases tubulointerstitial fibrosis.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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